Art. XV.—*The Anatomy of Some Australian Amphibia.*

Part I.

A.—The Openings of the Nephrostomes from the Coelom.

B.—The Connection of the Vasa efferentia with the Kidney.

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(With Plates XX., XXI.).

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INTRODUCTION.

The research, of which the following is a record, was suggested in the first instance by the conspicuous character of the Nephrostomes in the common "green" frog of Victoria, *Hyla aurea*, especially in connection with Professor Sedgwick's statement, so recently as 1905, of his doubt of the correctness of the descriptions of previous workers on the European and American genus, Rana. So far as I have been able to find, Rana, Bufo, Discoglossus, Bombinator and Alytes are the only genera of the Anura which have received any attention from workers in reference to the points herein discussed. The remainder of the Bufonidae, the Hylidae and the Cystignathidae, have been untouched heretofore. These three families are well represented in Australia, especially the Cystignathidae. I have therefore endeavoured to fill the gap by this work on the following eight forms:—

Hylidæ.

Hyla aurea. *H. lesueurii*

Bufonidæ.

Notaden bennetti. *Pseudophryne australis.*

CYSTIGNATHIDÆ.

Crinia signifera.	*Chiroleptes alboguttatus.*
Heleioporus pictus	*Limnodynastes dorsalis.*

In the consideration of the two points especially dealt with in this paper, a study of the general structure of the kidney and testis also becomes necessary.

A.—THE OPENINGS OF THE NEPHROSTOMES FROM THE COELOM.

The special interest of the Nephrostomes, or openings from the body cavity, in connection with the kidneys, as has previously been pointed out, lies in (1) the fact that they exhibit according to the findings of Marshall, Bles, Frankl and Farrington *(vide infra)* in the forms examined by them, a good example of transference of the structural relationships and function of an embryonic organ during development, and (2) the importance of determining the exact forms in which an organ present in the embryo loses its function or ceases to exist in the adult. That these nephrostomes are present in the larval Amphibian, as well as in the embryo of other groups, is well known: that they persist in the adult of many of the Fishes and of the Urodeles or "tailed-Amphibia," still with their embryonic relationship to the uriniferous tubules of the kidney is also an accepted fact, their function in this case being doubtless the passage of fluid material from the coelom to the exterior. Moreover it is just as certain that they do not normally persist in the adult of the higher Vertebrata. It becomes desirable then to ascertain just where these structures disappear as a feature of the adult, and what changes take place in their relationships and function during their disappearance.

Historical.

The history of the discussion as to the presence and relationships of the nephrostomes in the Anura is a very interesting one. I give it here in brief outline:—

1874. Heidenhain. [Ecker, pp. 327, 336] was unable to find them.

1875. Spengel. [Spengel, '77, and Marshall and Bles, '90, p. 147] stated that the nephrostomes open on the

surface of the kidney. He found them in Rana, Bufo, Bombinator and Discoglossus. There may be one nephrostome to two tubules, or one to four nephrostomes to one tubule. They are connected with the fourth part of the uriniferous tubule.

1875. Meyer. [Ecker, pp. 328 and 336, and Marshall and Bles, '90, p. 147]. Quite independently and unknown to each other, Meyer confirms Spengel's work. He found 250-360 in Rana.

1877. Nussbaum. [Farrington, '93, p. 309], confirmed previous work as to the internal opening.

1880. Nussbaum. [Ecker, p. 328, 336: Nussbaum, '80], stated that the nephrostome is connected with the neck of the tubule in the larva, but opened into the Renal Portal Vein in the adult.

Weidersheim, according to Haslam [Ecker, p. 336], at one time stated that the nephrostomes had no openings at all on the surface.

1886. Nussbaum and Wichmann. [Marshall and Bles, '90, p. 150]. These found that in Rana fusca, R. esculenta, Bufo calamites, and Alytes obstetricans, they open into the Renal Veins and so to the Inferior Vena Cava.

1886. Hoffmann. [Hoffman, '86], asserted that they end blindly in the adult, though connected with the neck of the capsule in the larva.

1886. Wiedersheim. [Wiedersheim, 86, p. 756], accepts Nussbaum's work of 1886 with the remark that the peritoneal fluid is no longer lost, but is returned to the general circulation like the rest of the lymph.

1889. Haslam. [Ecker, p. 336], states that he could not find any trace of them, and that if present (1) they are very difficult to find, (2) they do not form a free communicating path between any part of the uriniferous tubules and the abdominal cavity, and (3) their superficial terminations have no free cilia

1890. Marshall and Bles. [Marshall and Bles, '90, p. 133]. They are easily seen, though not in every section in a series; also, the whole length of a nephrostome is rarely seen in one single section. The nephrostome-tubule has no relation except of apposition with the urinary tubules, and opens by a conspicuous aperture through which a tuft of flagella projects into the Renal Vein.

1893. Farrington. [Farrington, '93], states that in Rana catesbiana, and R. virescens, they may open directly inwards, or take a short horizontal first. He could not trace the internal opening with certainty: though ciliary action was seen at the external openings. By injection, he obtained almost conclusive proof of their connection with the Renal veins near the ventral surface.

1898. Bles. [Bles, '98], finding considerable scepticism regarding the point, exhibited before the Cambridge Philosophical Society 4 sections, "showing a nephrostome tubule opening into a narrow space lined with endothelium and containing a blood-corpuscle, the space being continuous with venous spaces in neighbouring sections."

1898. Frankl of Vienna. [Frankl, '98], attacking an allied problem finds incidentally by injection that there is no connection between the nephrostomes and urinary tubules.

1898. Beissner. [Beissner, '98], confirms the statements of Nussbaum [1886] Marshall and Bles.

1902. Marshall's "Frog." In the 8th edition of this work, the Editor confirms and accepts Marshall's and Bles' findings in 1890 and 1898, i.e., that the nephrostomes open into the Renal Veins.

1902. Howes. [Howes, '02, Pl. VII., Figs. XXXV., and XXXVI.], shows clearly the opening into these blood-vessels.

1905. Sedgwick, in the new edition of his text-book of Zoology ['05, p. 295], writes: "In the Anura nephrostomes are present. . . . It has been

asserted that they open into the Renal Veins. This statement must be accepted with caution. It appears more probable that they have lost their connection with the renal tubules, and persist as ciliated cups on the surface of the kidney."

1906. Holmes, ['06, p. 204] accepts the internal opening of the nephrostomes as into the branches of the Renal Vein.

It was then with the hope that our Australian forms might throw some light on this problem, that this part of the work has been done.

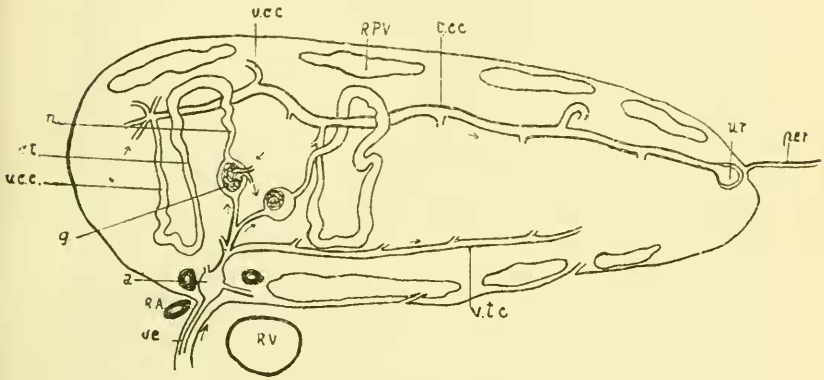
Structure.

In general external form and position, the kidneys of the forms examined do not differ materially from those of Rana, being flattened bodies, three to four times as long as they are broad, and one-third to one-quarter of their width in thickness. [See Pl. XX., fig. 1.] Situated just ventral to the dorsal body wall, in the abdominal lymph space, they are covered ventrally by the peritoneum which keeps them in position against the dorsal body wall. The ventral surface is generally flat or occasionally distinctly concave, while the dorsal surface is always more or less convex. The outer edge of each kidney is formed by the ureter which arises by branches in the substance of the kidney, and runs back behind the kidney dorsal to the large intestine, to open into the roof of the cloaca.

Blood-vessels.—The Renal Arteries vary in number, being generally in 5 or 6 pairs. They enter the kidney usually at about one-third of its width from the inner edge, and break up at once into numerous branches. Somewhat dorsal to the ureter runs the Renal Portal Vein often receiving one or more lumbar veins from the body wall. This vein breaks up into numerous branches running inwards across the dorsal surface of the kidney, breaking up as they do so. The Renal Veins arise on the ventral surface of the kidney, sometimes nearer to the inner edge than the entrance of the Renal Arteries (e.g., in Crinia signifera). More often these veins leave the kidney on the outer side of the arteries (e.g., in Hyla aurea, Pseudophryne australis, Heleioporus pictus, Notaden bennetti, and

Chiroleptes alboguttatus), in which latter they arise from the middle of the kidney. In a few forms, these veins are very short, the kidneys being so closely apposed as to appear as one mass ventrally, as in Pseudophryne australis and Notaden bennetti. Rarely the kidneys are distinctly unequal in length as in Chiroleptes alboguttatus, where the right kidney is fully 2 mm. longer than the left.

In the more detailed structure of the kidney, considerable variations are found.



Scheme of Amphibian Kidney seen in transverse section—to show the relationships of the male reproductive ducts and the uriniferous tubules.

a. = Ampulla on longitudinal Bidder's canal.
c. t. = Ciliated part of uriniferous tubule.
g. = Glomerulus of Malpighian body.
n. = Neck of uriniferous tubule.
per. = Peritoneum.
R. A. = Renal Artery.
R. P. V. = Renal Portal Vein.
R. V. = Renal Vein.
t. c. c. = Transverse collecting canal.
ur. = Ureter.
v. c. c. = Vertical collecting canal.
v. e. = Vas efferens.
v. t. c. = Ventral transverse canal.

HYLIDAE.

Hyla aurea.

This may be taken as the normal type. [See Pl. XX., fig. 1.] In transverse section the kidney is more or less triangular, the base being towards the middle line of the body. The branches of the Renal Veins occupy much of the outer part of the ventral side, the Renal Arteries entering nearer the inner edge of the kidney. The peritoneum is continued dorsally on the kidney for a short distance from the outer edge, but leaves the kidney well before it reaches the inner edge of the ventral surface. An "adrenal body" is present on the ventral part of each kidney.

The fibrous connective tissue supporting the tubules and blood-vessels is present here, to much the same extent as in Rana, where it has been known as "kidney-parenchyma" by some German writers [Cf. Pl. XXI., fig. 3.] The Malpighian bodies are often very much elongated, but not always, their greatest length being found in the inner part of the kidney. They form a more or less irregular layer in the upper part of the ventral half of the kidney. The neck emerges from the dorsal part of the Capsule, and runs more or less vertically upwards. The blood-vessels enter and leave the side of the glomerulus. There are no special points of difference in the microscopic structure of the tubules calling for comment— the pavement epithelium of Bowman's Capsule, the ciliated cubical epithelium of the neck, the large-celled convoluted portion often pigmented, and the collecting tubes with their wider cavities and more or less cubical epithelium, being very similar to corresponding parts found in other forms such as Rana.

Under favourable conditions, there may be seen under a hand-lens numerous minute pit-like structures on the ventral surface of the kidney. These are the external openings of the nephrostomes. These funnel-shaped depressions are situated chiefly on the inner half of the ventral surface of the kidney, and pierce the peritoneum which is loosely attached to the kidney wall. The walls of the "funnel" are formed of large cubical flagellated cells, with round, sharply defined nuclei—the flagella are always directed inwards, away from the surface of the kidney. [Cf. Pl. XXI., fig. 3.] Throughout this paper, the

word "cilia" will be used instead of "flagella" for convenience —though the latter is undoubtedly more correct.] In number the nephrostomes vary considerably—from 150 to 200—[Cf. Rana catesbiana with 150 at most, and R. esculenta with 250 to 360, Farrington, '93]. In diameter they average in this form 0.04 mm., i.e., somewhat larger than in Rana; in length or depth, the funnel averages 0.09 mm. In H. aurea we occasionally find long branched ciliated tubules present as direct internal prolongations of the funnels, these run parallel to the surface, or at other times towards the centre of the kidney for about one-third of its thickness, from the ventral edge. I have not been able to detect any division or union of these finer tubes, such as has been described by Spengel in Rana [Spengel, '77, p. 330]. Not infrequently, a large funnel is seen close to the outer edge of the kidney where the parietal peritoneum leaves the kidney to become attached to the body-wall.

The effects of the activity of their cilia may sometimes be seen on the surface of the kidney, in the currents set up by their movement, e.g., when the living kidney is placed in salt solution containing finely divided carmine. In such a case, in H. aurea, I have seen undoubted though small movement of the suspended particles of carmine, all external source of movement having been carefully eliminated—although Haslam and Farrington state that they have been unable to detect any such evidence of ciliary movement, in the forms examined by them.

Their internal relations are by no means easy to make out, owing to two circumstances. Not only must the internal opening (if such exist) be very minute, else the corpuscles may be forced through it outwards, but it is also extremely likely that even if it be not collapsed at death, it will contract during fixation. After the examination, however, of numerous complete series of sections, amounting to many thousands in number, there is not the slightest doubt as to the existence of an internal opening [Cf. Pl. XXI., fig. 3], and that this leads into the Renal Veins, or into blood spaces directly continuous with these veins, the long cilia protruding into these cavities among the corpuscles much in the same way as in Rana [Bles, '98, p. 75; Howes, Pl. VII., Figs. XXXV., XXXVI.]. The actual internal opening has only been found in other forms among the

Anura, so far as I am aware, by Nussbaum and Wichmann, Marshall and Bles, in the genera Rana, Bufo, Bombinator, and Alytes. Several others speak of the close relationship of the internal end with the blood-vessels, but state that the opening could not be traced with certainty. In no case, however, is there any semblance of a connection with the Renal tubules.

In order to further test the truth of this observation, various experiments were made. In the first of these, a modification of Nussbaum's method, carmine was injected into the body cavities of freshly pithed frogs, so that the carmine might if possible be taken in through the ciliated funnels. Upon examination, the carmine was found to have entered the kidney by these openings and to be present only in the blood-vessels of the ventral surface, in the Renal Veins, and in the Posterior Vena Cava. In the second experiments, carmine was carefully injected into the Renal Portal Vein of a freshly-killed frog, an opening being made in a branch of the Truncus Arteriosus. After proper fixing, staining, embedding and sectioning, the carmine was found to be present in all the venous spaces of the kidney, some having escaped under the considerable pressure exerted, through the nephrostomes, the particles being entangled among the cilia of these funnels. But I was not able to find any carmine within the kidney tubules. Farrington ['93, p. 312] found considerable difficulty in preventing the carmine particles from being scattered by the knife, through every part of the kidney; but in these experiments of mine, no such difficulty appeared, as the carmine was present in such a manner in the blood spaces as to leave no room for doubt as to the method of its distribution.

Hyla lesueurii.

The general relations of the kidney are as in H. aurea. The adrenal body is very well developed.

The nephrostomes are comparatively few in number, averaging about 30 in each kidney. Here also I have been able to detect an internal opening from the nephrostomial funnels into the blood spaces on the ventral surface of the kidney. This observation is confirmed by the results of injection. After injection of the body cavity of freshly-pithed frogs as previously

described for H. aurea, the carmine particles were found in the nephrostomial tubes and throughout all the blood-spaces of the kidney and in the Renal and Renal Portal Veins, in which the particles were embedded in the mass of coagulum, but none in the uriniferous tubules or ureter. Apparently the pressure in the Posterior Vena Cava was so great in this instance that the carmine found it easier to spread back into the branches of the Renal Portal Vein than to pass on into the Posterior Vena Cava.

Bufonidae.

Pseudophryne australis.

The kidneys in this form are much more triangular in transverse section than those of Hyla aurea, the outer edge being formed by the ureter ventrally and Renal Portal Vein dorsally. The Renal Veins emerge at the ventral edge of the inner side, while the Renal Arteries enter the kidney just internal or dorsal to the exit of the Renal Veins, and the Vasa efferentia enter immediately to the outer side of these veins.

The general arrangement of the uriniferous tubules seems to be as in Hyla aurea, the difference in character between the glandular and conducting parts of the tubules being specially well-marked. The Malpighian bodies are almost spherical and somewhat less numerous than in Hyla aurea. There is but little supportive fibrous tissue, though the blood-spaces are still small and normal in relationship. The nephrostomes are most numerous posterior to the plane of the hinder end of the Testes, and from the median line of each kidney outwards. Their funnels run more lengthwise and obliquely in the kidney in this form than in the previous forms, so that they are less often cut longitudinally in transverse sections of the kidneys. However, here, as in Hyla aurea and H. lesueurii, they open into the blood-spaces directly connected with the Renal Veins, their internal ends being always surrounded by a mass of blood corpuscles.

Notaden bennetti.

The kidneys of this form show the same tendency to adpression of the inner part of their dorsal surfaces as has already been found in Pseudophryne australis. Here also the Renal

Veins are short and enormously large, causing often deep depressions on the ventral surface of the kidney. The ureter in some specimens of this species lies right outside the kidney in the parietal peritoneum. Seen in transverse section [see Pl. XX., fig. 2], especially in the posterior half, the kidney of Notaden is conspicuously unlike any of the forms so far described. The vertical disposition of the tubules is very strongly marked, in places forming radiating lines from the midventral line of the kidney. The tubules have often pigmented walls. There is practically no "kidney-parenchyma," the whole kidney being extremely vascular, more so than in any other form of which I have any knowledge, though Chiroleptes alboguttatus, and Heleioporus pictus are also remarkably vascular. The extreme posterior end has comparatively small blood-spaces, but they increase very rapidly in size and number forwards from this point. Along the midventral line of each kidney is developed as a core or "pelvis" occupying one-third to one-half the thickness of the kidney, a series of large venous spaces traversed or subdivided by a network of trabeculae, the blood-spaces in which are connected on the one hand with the Renal Veins, and on the other with the radiating blood-spaces of the general kidney-substance. The general appearance of the kidney microscopically is that of a groundwork of corpuscles in which the tubules and Malpighian bodies are embedded. The Malpighian bodies are normal in number, round and somewhat small in comparison with the size of the kidney. Those in the outer half are often quite close to the ventral surface of the kidney, while those elsewhere form two or three irregular rows at about the middle of the kidney thickness. The differences in structure and appearance between the necks of the Capsules, the conducting, glandular and collecting tubules, though similar in character to that found typically as in Hyla aurea, are very much more strongly marked. The nuclei of the cells forming the necks, and the conducting tubules stain very deeply indeed with nuclear stains, so that it is only by careful tracing of the tubules along their length that one can believe that these parts and the glandular parts are really connected.

In nephrostomes also, Notaden bennetti is quite unlike previously-described forms. They are extremely numerous pos-

teriorly where the venous spaces form nearly half the thickness of the kidney, and diminish in number somewhat irregularly towards the anterior end. In one kidney alone I counted 1067 external openings of nephrostome funnels: I have seen as many as 10 external openings in a single thin section across one kidney. There are here several totally different types of nephrostomes. The first are the normal ones like those found most frequently in Hyla aurea, which are wide, short and unbranched, and run almost horizontally beneath the kidney surface, and emptying directly into the main venous spaces, very much like that figured for another form in Fig. 4, except that there is no supporting tissue in Notaden bennetti. The second set, although resembling some of those in Hyla aurea in that they branch, are quite distinct from those in the structure of the "funnel." This is long, narrow and more tubular than in any form previously described: it branches freely, running a considerable distance into the centre of the kidney. The branches, of which there may be as many as five from one nephrostome, run generally along the trabeculae and then leaving them, end in a blood-space. There I believe them to open, though I have not been able to detect the actual aperture. These nephrostomes are especially numerous on either side of the main venous space.

Just within the inner and outer edges of each kidney, especially in the outer edge, are here and there coils of small thin-walled tubes, whose cells have deeply staining nuclei, resembling generally the second or branching type of nephrostomial-tubule. Sometimes these open clearly to the exterior—some even on the dorsal side of the outer edge and anteriorly (though still through the peritoneum which often is continued on to the dorsal side of the kidney for a short distance)—at other times they do not open, but are still connected with the surface of the kidney, and may end blindly internally in a swollen mass of cells—or, one, two, or three nephrostomes may open into a single uriniferous tubule in its 4th part—or, yet again, may apparently come into relationship with a smaller type of Malpighian body than that usual elsewhere, while in yet others, the Malpighian body is still there, but is very degenerate.

It is, I think, evident that here we have exactly what Spengel [Spengel, '77] and Meyer have described in Rana, viz., that the nephrostomes open into the 4th part of the uriniferous tubules, and that two or more funnels may open into one tubule and vice versa. It is curious that after 20 years, during which time no one has confirmed Spengel's and Meyer's work, but on the contrary everyone has shown it not to be true in the generality of cases, one should come across a similar condition evidently as a passing stage in a form such as Notaden belonging to quite a different group of the Anura.

Forms of Notaden bennetti have been examined from New South Wales as well as Central Australia. The description above given refers to the Central Australian form. There is no comparison in the amount of blood supply in the two sets of forms, the New South Wales form being practically normal as regards its general vascularity, though there is still a lack of supporting tissue, and a tendency to a central arrangement of large venous spaces such as are so marked in the Central Australian form of Notaden, and to a less extent in Heleioporus, as will be seen later. It should be added, however, that in the New South Wales, as well as the Central Australian forms, the various types of nephrostomial tubules are present, although the total number of external nephrostome openings is very much less.

Notaden, it may be remembered, is one of the burrowing forms met with frequently in Northern Central Australia, where during the drought season they remain underground, in permanent burrows, having first filled themselves out with water [Spencer, '96, pp. 159, 163, etc.]. This water is apparently taken in through the mouth, and probably through the skin also, during the time of plentiful water, being then absorbed into the vascular system, and excreted by the kidneys, passing into the urinary bladder. It will be found that in the Report of the Horn Expediton [loc. cit.] Professor Baldwin Spencer has described this water as being in the body-cavity of these frogs, but he informs me that on subsequent visits to Central Australia and dissection of a considerable number of forms, he has discovered that it is stored in the urinary bladder and not in the body cavity. In Notaden bennetti there is always a con-

siderable amount of coagulum along the ventral surface of the kidney, showing the presence of considerable lymph in the body cavity also. How the presence of so much water in the bladder is related to the tremendous development of the nephrostomes in this form is not at all easy to see, though there is certainly an intimate relationship between the two facts. It seems most probable that the water from the extremely thin-walled bladder soaks out into the body-cavity, and is passed back by the nephrostomes into the blood vascular system whence what is required may be taken by the organs of the body, the surplus being again excreted into the bladder, and so on; thus maintaining a constant circulation of this water for the benefit of the body generally. This return of waste with the water from the bladder would be less injurious than in the ordinary frog, since in these aestivating frogs oxidation of the tissue will be at a minimum, probably only sufficient to maintain life.

Cystignathidae.

Crinia signifera.

The general kidney arrangement does not call for any special comment, the relationships of the kidney tubules, blood-spaces and supportive tissue resembling those found in Hyla aurea. The glomeruli are spherical, very few in number, and situated close to the ventral surface. The nephrostomes also are very few in number, what there are being chiefly at the anterior end. Their walls, however, are very easily distinguishable from those of the uriniferous tubules. They are often not much more than a slit, in some cases no cavity or cilia being visible, but wherever determinable, they open into the venous blood-spaces on the ventral surface.

The body cavity of this form also was injected, with the result that the carmine was drawn through the nephrostomes into the blood-spaces of the ventral one-third of the kidney, though to a very much less extent than in other forms similarly treated. The small number of nephrostomes, their frequently diminished cavity, and their apparently smaller functional activity would seem to indicate that in Crinia signifera, they are rapidly losing

their function and ceasing to exist, compared with other forms herein described, unless possibly Hyla lesueurii.

Chiroleptes alboguttatus.

This form, like Notaden bennetti, is a burrowing one which stores up water in its body while aestivating. Its kidneys are almost oval in transverse section, and seem peculiarly liable to be folded back against one another, their inner edges with the Renal Veins forming the ventral edge of the mass (Cf. also Pseudophryne australis, and Notaden bennetti). The dorsal surface of the kidney is the more convex. The kidneys resemble those of Notaden in having the minimum of fibrous tissue and very large blood-spaces, though the large central venous space found in Notaden is lacking here, the Renal Veins arising in the usual way in Chiroleptes alboguttatus. The general vertical (dorso-ventral) arrangement of the tubules and blood-spaces is very strongly marked as seen in transverse sections, the tubules being much pigmented and the blood-spaces crammed full of corpuscles. The Malpighian bodies are rounded, very few in number, and remarkably small in comparison with the size of the kidney. Indeed one often comes across sections in which no sign of Malpighian bodies is to be seen. They are found in the ventral one-third of the kidney thickness. The nephrostomes, on the other hand, are numerous and well-developed, though not nearly to such an extent as in Notaden bennetti. In number I found in one kidney, 210 external openings, the number diminishing from the anterior end backwards. There is hardly a section in a full series through the whole length of the kidneys, in which the nephrostomes are absent, while there may be as many as six in one section. To a certain extent they resemble Notaden in having two kinds of "funnels," though the branched forms are much less developed than in Notaden. These slope inwards as a rule, at an angle of 20 to 30 deg. for a short distance, and then branch: the branches coil more or less through the substance of the kidney, but always end in blood-spaces, where their cilia protrude among the corpuscles which are so densely packed around these internal openings. There is also, near the median edge of the

kidney a series of large short nephrostomial funnels which open immediately without branching into the main Renal Veins.

The strength of the blood pressure in the kidney, as well as a corroboration of the connection of the funnels with the blood-spaces, is shown in the fact that in two or three cases the red corpuscles had been forced through the internal opening of the nephrostome funnel, and were lying entangled among its cilia. As may be inferred from this statement, the cavity in many of these funnels is much greater than in some others of the previous genera. Here, too, although the development of nephrostomes is not so great as in Notaden bennetti, the association of intense vascularity of the kidney with aestivation is very evident.

Heleioporus pictus.

Here also the kidneys are almost oval in transverse section. The Ureter and Renal Portal Vein lie on the dorsal surface, near but not at the outer edge. Heleioporus pictus is another of the burrowing aestivating forms, and, as in Notaden bennetti and Chiroleptes alboguttatus, we have here a very vascular kidney somewhat resembling Notaden in type, but much less developed. As in those forms also, the connective tissue is very small in amount, and the blood-spaces are so crammed full of corpuscles that no definite walls are often to be found. The regular dorsal-ventral arrangement of the kidney is interfered with somewhat by the greater convolution of the glandular part of the kidney tubules. The glomeruli are spherical and much more numerous than in the last two forms. The neck of the tubule opens dorsally from the Malpighian body, while the blood vessels enter and leave the outer side of the glomerulus [see Text figure and Pl. XXI., fig. 4]. The nephrostomes are not as numerous as in the last numbering in each kidney 105. They are almost entirely absent at the anterior end, gradually increasing in number to the beginning of the posterior one-third of the length of the kidney, and then diminishing very rapidly to the posterior end. They have been found to open some into the general venous blood-spaces of the kidney, where their cilia may be seen protruding inwards and surrounded by blood corpuscles: others lying on either side of the main branches of the Renal Veins

may open directly into them. The funnels are large and long [see Pl. XXI., fig. 4], and in the case of the former, which are branching forms, after entering the kidney obliquely they run horizontally for some distance and then branch, their branches running along the trabeculae far into the ventral half of the kidney thickness. These branching forms of nephrostominal tubules resemble those of Hyla aurea, and Chiroleptes albogut-tatus, rather than the more strongly defined type found in Notaden. I have examined specimens from Central Australia and from Victoria, and find very little difference in the kidneys of the forms from the two areas.

Limnodynastes dorsalis.

The kidneys are here much flattened ventrally and convex dorsally, the adrenal body forming in transverse section a conspicuous structure along the middle of the kidney. In general the internal structure is very similar to that found in Hyla aurea, the connective tissue being considerable in amount [see Plate XXI., fig. 3], and the blood-spaces small and empty and well-defined compared with those of the last three forms—the glomeruli are round and fairly numerous. The nephrostome funnels are short and unbranched and somewhat larger than in Rana catesbiana (.035 mm. in diameter according to Farrington ['93, p. 310]), while those of Limnodynastes dorsalis are .037 to .04 mm. They have a well-marked cavity, their internal ends projecting into the blood-spaces [Pl. XXI., fig. 3] among the corpuscles when these are present. I have not detected any funnels opening into the main branches of the Renal Veins as in some forms previously described herein. In specimens injected from the Renal Portal Veins under pressure, the carmine was found to be present throughout the blood-spaces, and had been forced out by the pressure into the funnels where the particles were found entangled among the cilia.

B.—The Connection of the Vasa efferentia with the Kidney.

Here, as in Part A, the object in view is to find a sequence of forms in this case illustrating the manner in which in the course of the evolution of the group, the male reproductive ducts have

been gradually separated off from the kidney tubules. In the Fishes and in the Urodeles and Coecilidae, the male reproductive ducts are very closely connected with the anterior sexual part of the kidney. In Bufo and Rana esculenta, they are closely connected with the Malpighian bodies of the urinary tubules of the kidney; in Rana fusca the connection is less close, being only with the collecting tubules. The severance increases in Bombinator and Discoglossus, till in Alytes the male ducts open quite independently of the kidney, into the ureter: i.e., a portion of the mesonephric duct separates off as a duct for the testis, and at the level of the wider end of the kidney this joins the remnant of the original mesonephric duct which functions as a kidney duct.

The question of the relationships in Rana has been a much vexed one from the time of Bidder's work in 1846, but as most of it has arisen through the confusion of the two species R. esculenta and R. fusca, its results may be summed up as above. Nussbaum's work ['97, p. 425.], and that of Beissner ['98, p. 168.] practically settle the main connections as given above for these two species, the only variation between the two being that Nussbaum has only found the longitudinal Bidder's canal in R. esculenta, while Beissner describes it in R. fusca also.

General Structure and Relationships of Ducts.

The testes lie ventrally to the anterior portion of the kidney, being kept in position by the mesorchium, the fold of peritoneum which encloses them entirely, except for one part of their inner surfaces where the blood-vessels and ducts enter or leave them. [See Pl. XX., fig. 1.] In shape and size, they vary greatly in different individuals and at different times of the year—and as most of my material was spirit-preserved, except Hyla aurea, Crinia signifera and Limnodynastes dorsalis, due allowance has to be made for distortion by pressure of other organs.

HYLIDAE.

Hyla aurea. [Pl. XX., fig. 1].

When fully developed, the testes are long, whitish cylindrical bodies, each end being rounded. They average 10 or 11 mm. in

length and 3 mm. in diameter. The Vasa efferentia run straight from the testis to the kidney, and then entering the latter spread directly dorsalwards, branching to enter the ventral ends of the long Malpighian capsules, as in Bufo [Spengel, '77] and Rana esculenta [Nussbaum, '97, 1 and 2, and Beissner, '98]. These canals have narrow cavities, and thin walls of small cubical cells with large darkly staining nuclei, and, as a rule, they stand out conspicuously in sections across the kidney of this form. I have not been able to make certain of the existence of a Bidder's canal in Hyla aurea, comparable to that described for Bufo cinereus by Spengel ['77], and by Nussbaum ['97, p. 425] for Rana esculenta, and by Beissner ['98, p. 168] for R. fusca also. There is no doubt, however, that the vasa efferentia do open into the Malpighian Capsules, unlike R. fusca, Bombinator, Discoglossus, and Alytes [Wiedersheim, '86 p. 784], where they open either into the collecting tubules (Cf. R. fusca), or into the ureter itself. As the testis in all male specimens examined by me was comparatively little developed, and no spermatozoa were present in the vasa efferentia, either inside or outside the kidney, it is possible that the Bidder's canal may be present, but small and contracted, and so evade recognition. It is chiefly the Malpighian capsules near the inner edge of the kidney which are thus connected with the vasa efferentia.

Bufonidae.

Pseudophryne australis.

Here the testes were large, flat, irregular in outline, together hiding fully two-thirds of the kidneys when viewed from the ventral surface, extending also anteriorly and laterally beyond each kidney. Here, as in all other forms of which male specimens were examined, the Vasa efferentia of each testis run in the mesorchium dorsalwards to the kidney of its own side. They then in Pseudophryne, enter the kidney at the outer edge of the Renal Veins without any previous branching. They appear to run straight in and without forming a Bidder's canal enter the Malpighian capsules at their ventral edge as in Hyla aurea. Curiously, although the testes in the specimens examined are large and well-developed, I could find no sperm

in the vasa efferentia, the kidney or ureter. I have, however, no doubt as to the connection of the branches of the Vasa efferentia with the Malpighian capsules as above described. [Cf. Pl. XX., fig. 1.]

Notaden bennetti.

In the male specimens of this species available, the testes were spherical bodies having about the same diameter as the kidney itself, but quite unsymmetrically placed—the one at about the middle of the length of its kidney, the other at the posterior end of its kidney—each lying laterally to the kidney of its own side, the mark * [in Pl. XX., fig. 2] indicating the inner edge of the testis lying in the mesorchium. As stated in Part I. A., the ureter lies often in the peritoneum lateral to the kidney, and often, near the hinder end of the kidney and posterior to this, it swells out to form a large glandular Vesicula seminalis. The Vasa efferentia pass in along the mesorchium, and entering the kidney run straight out to open into the Bowman's capsules of the Malpighian bodies in the inner one-third of the kidney. I have been unable to find any longitudinal Bidder's canal.

Chiroleptes alboguttatus.

In this form the testes are long and thin, and in the specimens examined very feebly developed, probably owing to the season of the year. They were approximately one-half the length of the kidney and one-third its average width. The Vasa efferentia pass into the kidney at its inner edge from the inner side of the testis as usual, and spread out at once into the kidney substance without forming any longitudinal canal. Apparently they enter the ventral part of Bowman's capsule, as in previous forms. Owing to the extreme vascularity of the kidney and the great number of corpuscles present in all the blood-spaces, it is difficult to make out the relationship of these ducts further than as indicated above.

Heleioporus pictus.

The testes of H. pictus are very irregular in shape, and unequally developed on each side—that on the one side being

nearly the full length of the kidney, and that on the other only half that length. The path followed by the sperm on its way to the exterior is more clearly seen here than in any other form examined by me, as not only were the testes very large and well developed, but the sperm could be traced right through the kidney to the ureter.

The Vasa efferentia leaving the testis run dorsally, forming a network in the mesorchium, to open into a longitudinal Bidder's canal. Both network and canal are continued posteriorly and also anteriorly to the plane of the testis. The canal lies further from the middle line than the Renal Arteries, and both are nearer the middle line than the Renal Veins. From the ampullae on this canal ducts pass both dorsally and laterally, dividing up greatly—one ventral branch runs ventrally towards the outer edge of the kidney forming the ventral transverse canal, as seen in the Text-figure. The branches of these canals open into the ventral part of the Malpighian Capsules. In many cases, this Bowman's capsule is greatly distended by the masses of sperm [See Pl. XXI., fig. 5], the glomerulus being pushed quite to one side. All the Malpighian bodies are not so connected with the sperm ducts, and there is certainly a relationship between the position of the Malpighian body and its connection or otherwise with the sperm ducts. Thus no sperm is to be found in the Malpighian bodies occupying the outer one-third of the width of the kidney even when sperm is present in the tubules near by. One is apt to be misled as to the existence of such connection, since it does not follow invariably that even when the sperm ducts, and the uriniferous tubules are both full of sperm, that any will be found in the Bowman's capsule to and from which they are clearly open. Nevertheless, I have not been able to find any sperm ducts opening into the Capsules along the outer edge of either kidney (Cf. previous forms, e.g., Hyla aurea, and Notaden bennetti). From the Malpighian bodies, the sperm passes by the ordinary uriniferous tubules into the transverse collecting tubes which run outwards parallel with the dorsal surface of the kidney to pass their contents into the ureter where the sperm may be seen in great numbers.

Lying in the coelom ventral to the kidney is a flat branching structure nearly coextensive in length with the testis on either side. It is attached to the mesorchium on the outer side of the latter, by connective tissue across which small arteries pass from the Renal Arteries, and small veins to the Renal Veins. In appearance it is somewhat lymphoid, and might be regarded as a rudimentary fat-body, this being absent in the specimens examined, but for its position. That structure is attached normally to the anterior end of the testis, while this lies between the kidney and the testis for almost the whole extent of the latter. A very rudimentary condition of what is apparently the same structure was seen in one instance in Notaden bennetti, where it is in much closer relationship to the kidney surface. I am unable at this stage to add any further evidence as to its homologies or function.

Limnodynastes dorsalis.

Here the testes are very similar to those of Hyla aurea, except that occasionally the testis may lie quite anterior to the kidney. The arrangement of the Vasa efferentia, of the sperm ducts in the kidney and their entrance into the Malpighian capsules, are all similar to that already described in previous forms. As in Hyla aurea, Pseudophryne australis and Notaden bennetti, I have not been able to find any longitudinal Bidder's canal, the Vasa efferentia apparently going straight into the substance of the kidney.

Summary.

The results of this enquiry may be briefly summed up thus:—

1. Nephrostomial openings from the coelom are present in each of the eight species examined—viz., Hyla aurea and H. lesueurii; Pseudophryne australis and Notaden bennetti; Crinia signifera, Chiroleptes alboguttatus, Heleiporus pictus and Limnodynastes dorsalis.

2. There are five main types of nephrostomes and nephrostomial tubules.

3. The first, which never branch, open directly into the main branches of the Renal Veins. These are present in all forms.

4. Those of the second type are unbranched nephrostomes opening into the uriniferous tubules, as first described by Spengel in Rana. These are found in Notaden bennetti only.

5. The third type consists of branched nephrostomial tubules opening into the venous spaces. These are found in all forms, and especially well developed in Notaden bennetti.

6. The fourth type is also branched nephrostomial tubes opening however into the uriniferous tubules. This is found in Notaden bennetti only.

7. The fifth type is a third form of branched nephrostome tube, which is closed at either or both ends. These are only known in Notaden bennetti.

8. It will thus be seen that in all forms, nephrostomes opening into the Renal Veins are present, these being the only type present in most; in one form Notaden bennetti, all five kinds of internal connections are found.

9. It appears evident that these structures are undergoing very rapid modification at the present time.

10. In Notaden bennetti and Chiroleptes alboguttatus, the vascularity of the kidney is very strongly marked—the uriniferous tubules appearing to lie in a series of much branched sinuses—the epithelial lining being in many cases almost impossible to determine. The same is true, though to a less extent, in Heleioporus pictus. The vascularity would thus appear to be associated with the capacity of these frogs for storing water in the urinary bladder while aestivating in their burrows during the dry seasons.

11. There is also a marked difference in the number of nephrostome openings in one kidney from a minimum of 30 in Hyla lesueurii, to a maximum of 1067 in Notaden bennetti.

12. Hyla lesueurii and Crinia signifera are the most degenerate in respect of their nephrostomes.

13. Evidence from frogs injected with carmine indicates that the nephrostomes do function for the conveyance of material from the body cavity into the kidney.

14. In all forms, the Vasa efferentia enter the kidney just external to the Renal Arteries.

15. In Heleioporus pictus a well-marked longitudinal Bidder's canal is present, as possibly also in Hyla aurea, though

not in Pseudophryne australis, Chiroleptes alboguttatus, Notaden bennetti, or Limnodynastes dorsalis.

16. In all forms examined the Vasa efferentia branch and enter the ventral part of Bowman's capsules, in the inner one-third or two-thirds of the kidney.

Conclusions.

It must be conceded that there is considerable evidence given by the forms herein described, that in the course of their disappearance in the adult condition during the evolution of the group, the nephrostomes have been subjected to well-marked modification their original connection with the kidney tubules being transferred to the Renal Veins, with a correlated change of function from the passage of fluid to the exterior from the body cavity, to that of lymph vessels. Moreover their degree of development seems to be to a great extent individual or characteristic of the species, varying greatly in harmony with their functional importance both in turn being associated apparently with differences in the habit of the animal. In Notaden bennetti for some reason or other, alongside the greater development in one direction, there seems to have been a check to the harmonious development of these structures in all parts of the kidney, since there are still present along the edges, nephrostomial tubules in various stages of modification, as to their internal connections.

In all the species here described, of which male specimens were obtained, we find that the separation of the male reproductive ducts from the excretory ducts has not yet begun, the condition being comparable to that found in Rana esculenta, the higher stage found in Rana fusca not being present in the Australian species so far examined. They are therefore far less specialised than are the corresponding parts in Alytes obstetricans, the most specialised known in the Anura.

I have again to thank Professor Baldwin Spencer for the use of the Biological Laboratory in the University of Melbourne, where this work has been done, and for the use of his collection of specimens, and those of the Biological Museum, as well as for much kindly interest and valued advice.

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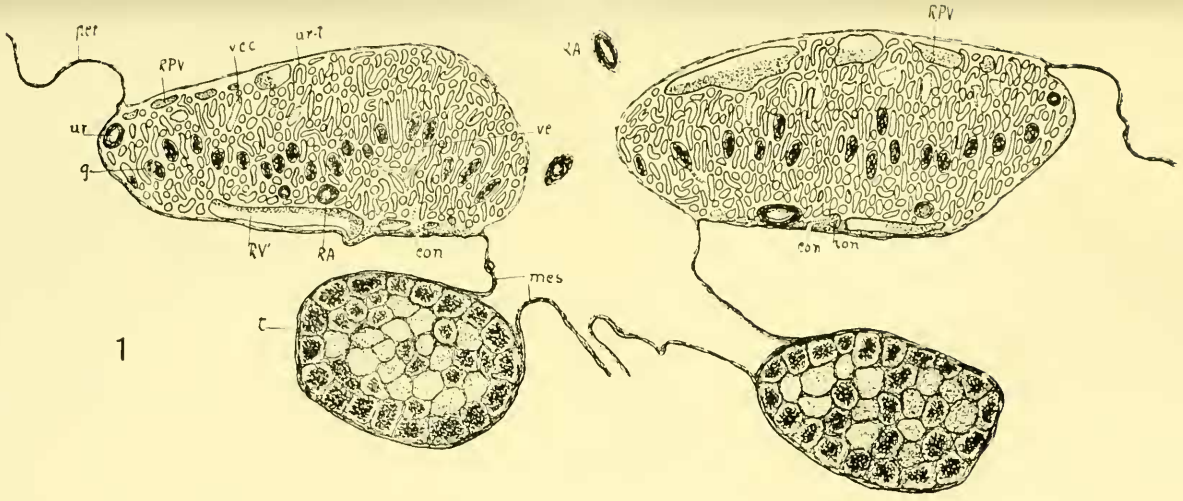
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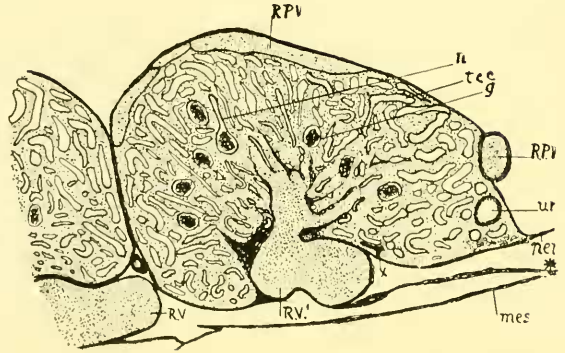
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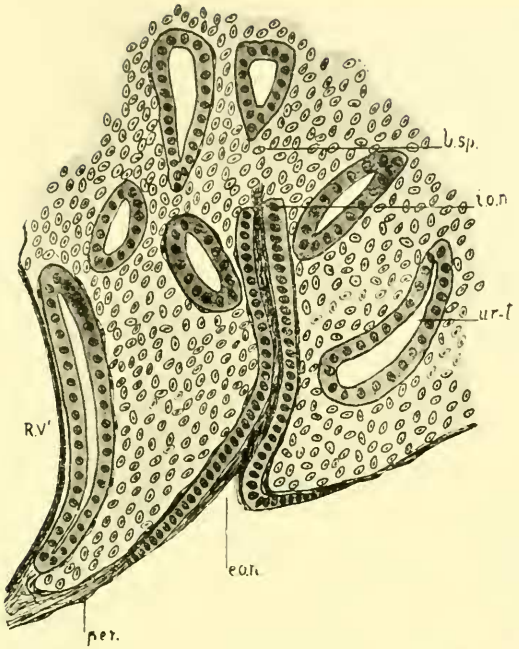
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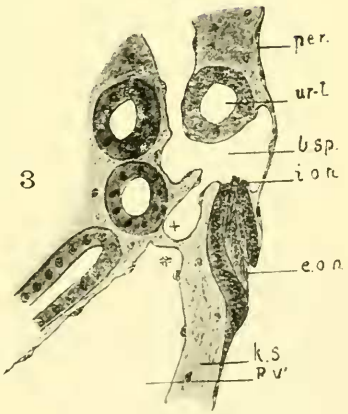
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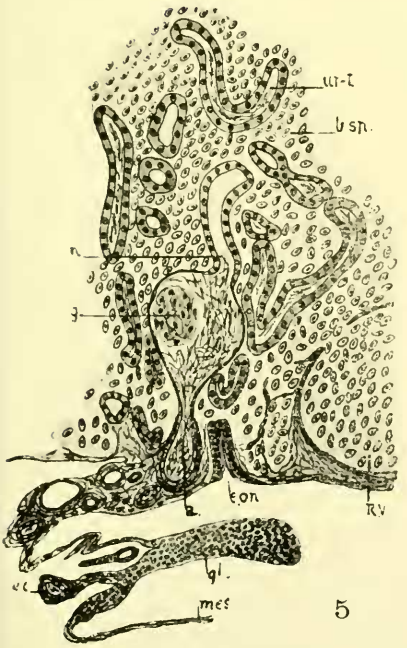
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